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Genomic Imbalances in Pediatric Intracranial Ependymomas Define Clinically Relevant Groups

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The outcome of pediatric ependymomas is difficult to predict based on clinical and histological parameters. To address this issue, we have performed a comparative genomic hybridization screen of 42 primary and 11 recurrent pediatric ependymomas and correlated the genetic findings with clinical outcome. Three distinct genetic patterns were identified in the primary tumors and confirmed by hierarchical cluster analysis. The first group of structural tumors, showed few, mainly partial imbalances ($n = 19$). A second numerical group showed 13 or more chromosome imbalances with a nonrandom pattern of whole chromosome gains and losses ($n = 5$). The remaining tumors ($n = 18$) showed a balanced genetic profile that was significantly associated with a younger age at diagnosis ($P < 0.0001$), suggesting that ependymomas arising in infants are biologically distinct from those occurring in older children. Multivariate analysis showed that the structural group had a significantly worse outcome compared to tumors with a numerical ($P = 0.05$) or balanced profile ($P = 0.02$). Moreover genetic group and extent of surgical resection contributed significantly to outcome whereas histopathology, age, and other clinical parameters did not. We conclude that patterns of genetic imbalances in pediatric intracranial ependymomas may help to predict clinical outcome. ***(Am J Pathol 2002, 161:2133–2141)***

Pediatric ependymomas are enigmatic tumors whose behavior is difficult to predict based on clinical and histological factors. These tumors are thought to derive from ependymal cells lining the ventricular system and fall into the broad group of gliomas.[1] Ependymomas comprise ~10% of all childhood intracranial neoplasms and with >50% arising in children younger than 5 years of age present a distinct management challenge.[2–4] In contrast to adults in which spinal tumors predominate, >90% of all pediatric ependymomas are intracranial in origin with most tumors arising infratentorially.[2,3,5]

Consistent histological grading of ependymomas has proven difficult and several different classification systems have been proposed.[3,6] The most frequently used system, World Health Organization 2000,[5] recognizes two principal variants in children: classic ependymoma (grade II) and anaplastic ependymoma (grade III). However, despite numerous studies the relationship between histological grading and tumor behavior remains unclear.[3,5–7]

The primary treatment for pediatric ependymomas is surgery and children who have a total tumor resection fare significantly better than those whose tumors are partially removed.[8,9] Infratentorial tumors are generally reported to confer a worse prognosis than their supratentorial counterparts,[3,9–11] but this may be because of their more inaccessible surgical location.[9–12] A young age at diagnosis is associated with an unfavorable outcome in some studies, but this may reflect a propensity for infratentorial location and concerns about the long-term morbidity of radiotherapy in young children.[2,4,9,13] Throughout the last 30 years survival rates for ependymoma have increased primarily because of improved surgical techniques and postoperative therapy.[8–13] However, this improvement lags far behind the advances made in other childhood cancers.[14] In part this is because of our poor understanding of the molecular pathogenesis of ependymoma. The characterization of tumor-specific molecular abnormalities that predict biologically favorable or unfavorable disease is important. Not only may it allow a more judicious use of current therapies such as radiotherapy, but it might also identify molecular targets against which new therapies can be directed. Genetic abnormalities are currently used to predict biological behavior in a number of pediatric cancers including neuroblastoma and rhabdomyosarcoma.[15,16] Similar biological correlates of tumor behavior are now

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required if we are to make improvements in the management of childhood ependymoma.

Although it is increasingly clear that genetic differences exist between adult and pediatric ependymomas,[17–20] there is still little information on the genetic differences within the spectrum of pediatric ependymomas and none relating genetic abnormalities to disease outcome. We have therefore performed comparative genomic hybridization (CGH) on a large retrospective series of pediatric intracranial ependymomas and correlated the genetic abnormalities with clinical outcome.

Materials and Methods

Patient and Tumor Samples

Forty-two primary and 11 recurrent formalin-fixed, paraffin-embedded pediatric ependymomas were obtained from Birmingham Children's Hospital, Birmingham, UK (38 tumors), Southampton General Hospital, UK (10 tumors), Bristol Children's Hospital, UK (3 tumors), and Newcastle General Hospital, UK (2 tumors). All tumors were centrally histologically reviewed before inclusion in the study to confirm a diagnosis of ependymoma according to World Health Organization 2000 criteria (DE, PR). Of the primary tumors, 24 were anaplastic and 18 were classic. Thirty-five tumors occurred in the posterior fossa and seven were supratentorial. The mean and median age of diagnosis was 63.76 and 51.5 months, respectively. Macroscopic total resection was achieved in 16 tumors from surgical report. Total resection was obtained in 5 of 7 supratentorial tumors and 11 of 35 infratentorial cases. Adjuvant therapy was administered to 37 children and consisted of radiotherapy, chemotherapy, or a combination of both radiotherapy and chemotherapy. The mean and median follow-up period was 53.81 and 38 months, respectively. Of 11 recurrent tumors, 9 occurred in the posterior fossa and 2 were supratentorial. Seven were of a classic histology, three were anaplastic, and two were unclassified.

DNA Extraction

Tumor DNA was isolated from 20 to 30, 10-μm-thick formalin-fixed and paraffin-embedded, tissue sections. The tissue was deparaffinized by incubation with xylene and disrupted in lysis buffer (1 mmol/L Tris base, 25 mmol/L ethylenediaminetetraacetic acid, 100 mmol/L NaCl, 0.5% sodium dodecyl sulfate, and 500 μg/ml proteinase K). A concentration of 500 μg/ml of Proteinase K (Sigma, Poole, Dorset, UK) was added to the samples twice a day. DNA was extracted by incubation with phenol (Sigma), recovered by precipitation with ethanol, and resuspended in TE buffer (Gentra Systems, MN). Normal reference DNA was extracted from human lymphocytes using the Puregene genomic DNA isolation kit (Gentra Systems, MN).

Comparative Genomic Hybridization

Tumor and reference DNA were nick-translated and directly labeled with Spectrum Green-2′-deoxyuridine-5′-triphosphate and Spectrum Red-2′-deoxyuridine-5′-triphosphate (Vysis, Downers Grove, IL), respectively. The volume of enzyme mix and incubation period were varied to give fragment sizes of 200 to 5000 bp as determined by gel electrophoresis. Eight hundred ng of tumor DNA, 400 ng of reference DNA, and 30 μg of Cot-1 DNA (Invitrogen, UK) were co-precipitated and resuspended in 3 μl of nuclease-free water and 7 μl of CGH hybridization buffer (Vysis). The probe mixture was denatured (75°C, 5 minutes) and hybridized to denatured, dehydrated male metaphase slides (Vysis) at 37°C for 72 hours. Slides were washed in 0.4× standard saline citrate/0.3% Nonidet P-40 (75°C, 2 minutes) and 2× standard saline citrate/0.1% Nonidet P-40 (room temperature, 30 seconds) and counterstained with 4,6-diamino-2-phenylindole (125 ng/ml) in anti-fade solution.

Image Analysis

Red, green, and blue images from representative metaphase spreads were digitized using a Cytovision (Applied Imaging, Santa Clara, CA) imaging system. Karyotypes from 15 metaphases were combined to produce a mean CGH ratio profile for each hybridization. Detection of imbalances was performed using Applied Imaging CytoVision High-Resolution CGH (HRCGH) software, which allows direct comparison of a mean CGH profile from a test hybridization with standard reference intervals produced from a series of CGHs using normal test DNA.[21] Regions where the mean CGH ratio profile, confidence limits, and standard reference intervals deviate from each other represent areas of genomic imbalance in the test specimen. A test:reference fluorescence ratio >1.5 was classified as a high-level gain.

The validity of our CGH technique using DNA extracted from archival specimens and analyzed using HRCGH software was initially tested by comparing CGH profiles obtained from archival tissue with those obtained from corresponding fresh tumor material. Eleven archival:fresh tumor pairs (nine adult and two pediatric ependymomas) showed 90% concordance when the relative imbalances of each chromosome arm were compared (99.5% confidence limits). Figure 1 shows concordant CGH profiles from two archival:fresh tumor pairs.

Conventional cytogenetic analysis was possible for eight pediatric ependymomas and the karyotypes were consistent with CGH profiles achieved using DNA extracted from corresponding archival tissue (cases 3, 11, 13, 25, 27, 28, 30, 39). Fluorescence *in situ* hybridization studies performed on paraffin sections from four tumors (cases 1, 6, 38, and 39), using a centromeric probe specific for chromosome 7 (CEP7, Vysis), also confirmed CGH data. In addition, loss of heterozygosity analysis with highly polymorphic markers at ~10 MB intervals along chromosomes 6q, 17q, and 22q, plus a marker at 11q13 for five tumors and matching constitutional DNA (cases 11, 12, 38, 39, and 43R) confirmed CGH data.

Figure 1. CGH profiles showing concordant results from two pairs of corresponding fresh (**left**) and archival formalin-fixed, paraffin-embedded (**right**) ependymoma specimens. CGH analysis performed using the High Resolution CGH software package (Applied Imaging, Santa Clara, CA). Chromosomal imbalances are recorded at regions where the average test:reference fluorescence ratio (**pink lines**) and 99.5% confidence intervals (**yellow lines**) lie outside the standard reference intervals (**black lines**) for a chromosome or chromosome region. A chromosomal loss is illustrated as a **red bar** to the immediate **left** of a chromosome ideogram and a chromosome gain as a **green bar** to the immediate **right** of a chromosome ideogram. **a:** Both fresh and paraffin samples show relative gains at chromosomes 16 and terminal Xq and relative loss at chromosome 22. **b:** Both fresh and paraffin samples show relative gains at chromosomes 5, 7, 9, 16, 17, 18, 19, and 20.

Statistical Analysis

Statistical analyses were performed using SPSS11. Survival curves were constructed using the Kaplan-Meier method and univariate comparisons were made by the log-rank test. Multivariate Cox proportional hazards regression was used to explore the effects of genetic and clinical factors on overall survival time. Association in two-way frequency tables was assessed by Fisher's exact test.

Results

Twenty-four of 42 primary tumors (57.1%) analyzed by CGH revealed chromosome imbalances with a mean of 3.2 imbalances per tumor (range, 0 to 25). Of a total of 135 abnormalities, 99 (73%) were whole chromosome imbalances, while 36 of 135 (27%) imbalances involved part of a chromosome or chromosome arm consistent with structural chromosome rearrangement. All chromosomes were involved in at least one imbalance with the most frequent changes being gains of chromosomes 1q (11 tumors, including 7 tumors with high-level gain of 1q), 2 (4 tumors), 7 (8 tumors), 8 (5 tumors), 9 (6 tumors), 18 (4 tumors), and 19 (4 tumors) and losses of chromosomes 3 (5 tumors) and 6 (4 tumors). A total of six tumors showed single chromosome abnormalities, three of which were gains of chromosome 1q. Table 1 details the CGH and clinical data for the primary tumors.

Preliminary examination of the CGH data revealed that unbalanced tumors appeared to form two distinct groups: tumors with many chromosome imbalances and tumors with relatively few chromosome imbalances. Hierarchical cluster analysis using the total number of imbalances together with the numbers of whole and partial chromosome imbalances as variables, defined two groups of unbalanced tumors:

Tumors with a total of 13 or more chromosome imbalances had between 11 and 20 whole chromosome im-

Table 1. Clinical Data and CGH Aberrations of 42 Primary Ependymomas

ID	Gender	Age at diagnosis*	Histology	Location	Resection	Adjuvant therapy	Censor	Follow-up*	CGH gains	CGH losses	Genetic group
1	M	48	AN	ST	T	RT	U	16	2, 7, 17q, 19	None	S
2	M	73	AN	ST	T	RT	C	182	1q, 3q24-qter, 17q	1p34-pter, 3p, 10q22-qter	S
3	F	58	CL	PF	P	RT	U	38	1q	None	S
4	F	103	CL	PF	T	RT	U	51	**1q,** 9p	None	S
5	F	67	AN	PF	T	CTRT	U	54	None	22	S
6	M	135	CL	PF	T	RT	C	43	7, 9, 11, **18,** 19	15q24-qter	S
7	F	57	AN	PF	P	CTRT	U	39	None	6	S
8	F	47	AN	PF	T	RT	U	42	**1q**	None	S
9	F	138	AN	PF	P	CTRT	U	22	**1q,** 9	None	S
10	M	50	CL	PF	P	RT	U	35	None	6q, 17p	S
11	M	45	AN	PF	P	CT	C	15	1q, 8, 9	None	S
12	M	152	CL	ST	P	CTRT	C	14	4, 7, 8	3, 11pter-q13	S
13	F	119	CL	PF	P	RT	U	49	**1q,** 18	1p31-p35, 20q	S
14	F	63	AN	PF	P	–	U	16	**1q**	None	S
15	F	71	AN	ST	T	RT	C	70	None	9	S
16	M	103	AN	PF	T	RT	C	25	1q	10q	S
17	F	54	AN	PF	P	RT	U	5	8	6q14-qter	S
18	F	150	AN	PF	P	None	C	1	17q	17p, X	S
19	F	53	AN	PF	P	RT	C	1	**1q**	2q24-qter	S
20	F	141	CL	PF	T	CTRT	U	52	1, 7, **8,** 9, 18, 19, 20, 21, 22q11.2-q12	10, 13, 14, 22q12-ter	N
21	F	128	AN	PF	T	RT	C	62	4, 5, 7, 9p, 13, 14	2, 3, 6, 8, 10, 15, 17, 18, 21	N
22	F	42	AN	PF	P	CTRT	C	220	2, **5,** 6, 7, 9, 10, 12, 17, **18**	1, 3, 8, 11, 13, 14, 15, 22	N
23	M	83	AN	PF	P	CTRT	C	33	Y, **1q,** 2, 5, 7, 8, 9, 10, **10p,** 11, 19p, 21	X, 1p, 3, 4, 6, 12, 13, 14, 16, 17, 18, 19q, 20	N
24	F	54	AN	PF	P	CTRT	C	12	1, 2, 7, 10, 11, 13, 19, 20	3, 5, 6, 8, 9, 15, 17, 21	N
25	M	16	AN	PF	T	CT	U	12	None	None	B
26	M	30	CL	PF	P	CTRT	U	43	None	None	B
27	F	31	CL	PF	P	CTRT	U	36	None	None	B
28	F	46	CL	PF	T	RT	C	69	None	None	B
29	M	29	CL	PF	P	CTRT	U	48	None	None	B
30	F	21	CL	PF	P	CTRT	C	38	None	None	B
31	F	33	CL	ST	P	CTRT	U	154	None	None	B
32	M	102	AN	ST	T	CTRT	C	223	None	None	B
33	F	26	CL	PF	P	RT	U	34	None	None	B
34	F	33	CL	PF	T	None	C	180	None	None	B
35	M	117	AN	PF	P	RT	U	65	None	None	B
36	F	41	CL	PF	T	RT	C	61	None	None	B
37	F	22	AN	PF	P	CTRT	C	139	None	None	B
38	M	17	AN	ST	T	CT	C	18	None	None	B
39	M	30	CL	PF	P	CT	C	16	None	None	B
40	M	14	CL	PF	P	RT	U	7	None	None	B
41	F	18	AN	PF	P	None	U	19	None	None	B
42	M	18	AN	PF	P	None	U	1	None	None	B

*Months; PF, posterior fossa; ST, supratentorial; AN, anaplastic; CL, classic; T, total; P, partial; CT, chemotherapy; CTRT, chemotherapy and radiotherapy; RT, radiotherapy; C, censored; U, uncensored. Gains shown in bold typeface represent high-level gains as detected by CGH (fluorescence test:reference ratio >1.5); B, balanced; S, structural; N, numerical.

balances and between 0 and 5 partial chromosome imbalances. These tumors were termed "numerical" ($n = 5$).

Tumors with a total of six or less imbalances had between 0 and 5 whole chromosome imbalances and between 0 and 6 partial chromosome imbalances. This group of tumors was termed "structural" ($n = 19$). A summary of imbalances observed in the structural and numerical groups is illustrated in Figure 2. Eighteen tumors in our cohort showed no genetic imbalances by CGH and these formed the "balanced" group.

Not only were different numbers of chromosome abnormalities observed in the genetic groups, but also specific chromosome imbalances were associated with particular groups. Gain of 1q was integrally related to the structural group in which 10 of 11 gains of 1q were observed. The pattern of whole chromosome imbalances in the numerical group was nonrandom with specific chromosome abnormalities observed frequently in this group—four of five of the losses of whole chromosome 3 were observed in the numerical group. Similarly gain of

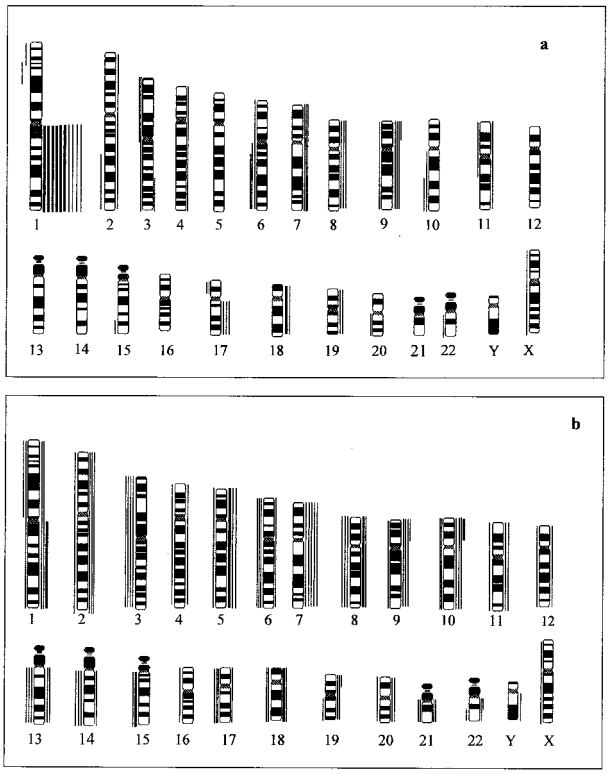

Figure 2. Schematic representation of CGH gains and losses in pediatric ependymomas. Each **bar** corresponds to genomic imbalance in one tumor. Gains and losses are shown on the **right** and **left** of each chromosome ideogram, respectively. High-level gains (CGH fluorescence ratio >1.5) are represented as **thick bars**. **a:** Structural tumors (n = 19) showing few mainly partial chromosome imbalances. **b:** Numerical tumors (n = 5) showing many, mainly whole chromosome imbalances.

whole chromosome 7 was more commonly observed in the numerical group in which five of eight gains of chromosome 7 occurred.

Fisher's exact tests were used to explore possible associations between genetic and clinical variables including tumor location, histology, extent of resection, adjuvant therapy, and age at diagnosis. All (14 of 14) children diagnosed younger than the age of 3 years showed a balanced genetic profile, conversely 24 of 28 children (86%) diagnosed older than 3 years of age showed either a structural or numerical genetic profile ($P < 0.0001$).

Univariate Kaplan-Meier survival analysis showed that structural tumors had a 5-year survival of 18%, which was worse than the 5-year survival figures of 67% for numerical tumors and 50% for balanced tumors, but this did not reach statistical significance ($P = 0.24$). To further assess the effects of genetic group on overall survival, multivariate analysis was performed using the additional covariates of tumor location, histology, extent of resection, adjuvant therapy, and age at diagnosis (>3 years *versus* <3 years). Structural tumors had a significantly worse outcome (adjusted 5-year survival, 10%) than both numerical (adjusted 5-year survival, 77%) and balanced tumors (adjusted 5-year survival, 55%) ($P = 0.05$ and 0.02, respectively) (Table 2, Figure 3). The extent of surgical resection also contributed to outcome in this analysis with partially resected tumors faring worse (adjusted 5-year survival, 15%) when compared with those that were totally resected (adjusted 5-year survival, 60%) ($P = 0.004$). Other factors did not contribute significantly to outcome. Multivariate analysis was repeated using genetic group as a variable for the posterior fossa tumors as these were our largest and most homogenous group. Again genetic group showed a significant effect on overall survival with structural tumors faring worse than either numerical ($P = 0.04$) or balanced tumors ($P = 0.02$).

Table 2. Adjusted Odds Ratios for Genetic Group in Intracranial Ependymomas from Multivariate Cox Proportional Hazards Regression Analysis Using Histology, Adjuvant Therapy, Extent of Resection, Age (<3/>3 Years), and Tumor Location as Variables

	Odds ratio	Significance	95% Confidence interval for odds ratio	
			Lower	Upper
Surgery	0.18	0.004	0.06	0.59
Structural *versus* Balanced	3.61	0.02	1.19	10.87
Structural *versus* Numerical	8.40	0.05	0.98	71.42

Patients receiving no adjuvant therapy, unknown adjuvant therapy, or chemotherapy alone were removed from this analysis because these groups were too small to be reliably analyzed (n = 11).

Because 1q was the most common specific chromosome abnormality in our cohort and was associated with a structural genetic pattern, both univariate and multivariate survival analyses were performed using gain of 1q as a variable. In univariate analysis, tumors with gain of 1q had a 5-year survival of 15% compared with 50% for tumors without gain of 1q although this difference did not reach statistical significance ($P = 0.45$). Similarly in mul-

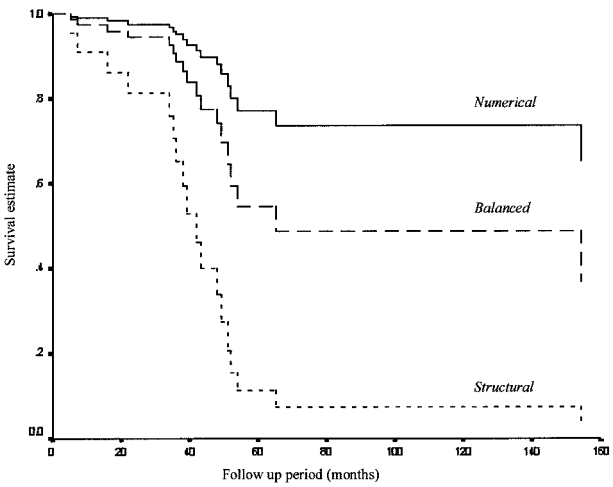

Figure 3. Adjusted survival curves for genetic group from multivariate Cox proportional hazards regression analysis using histology, adjuvant therapy, extent of resection, age (<3/>3 years), and tumor location as variables. The structural group is shown by a **dotted line**, the balanced group by a **dashed line**, and the numerical group by a **solid line**. Patients receiving no adjuvant therapy, unknown adjuvant therapy, or chemotherapy alone were removed from this analysis because these groups were too small to be reliably analyzed (n = 11). Structural tumors had a worse outcome than both numerical and balanced tumors ($P = 0.05$ and 0.02, respectively).

Table 3. Adjusted Odds Ratios for Genetic Group in Posterior Fossa Ependymomas from Multivariate Cox Proportional Hazards Regression Analysis Using Histology, Adjuvant Therapy, Extent of Resection, and Age (<3/>3 Years) as Variables

	Odds ratio	Significance	95% Confidence interval for odds ratio	
			Lower	Upper
Surgery	0.18	0.03	0.112	0.903
Gain 1q	2.5	0.1	0.785	8.2

Patients receiving no adjuvant therapy, unknown adjuvant therapy, or chemotherapy alone were removed from this analysis because these groups were too small to be reliably analyzed (n = 11).

tivariate analysis using the additional covariates of tumor location, histology, extent of resection, adjuvant therapy, and age at diagnosis (>3 years *versus* <3 years) there was no significant difference between tumors with gain of 1q *versus* tumors without gain of 1q (P = 0.22). Analysis of posterior fossa tumors using 1q as a variable revealed that tumors with gain of 1q had a trend toward a poorer overall outcome (adjusted 5-year survival, 5%) compared with tumors without gain of 1q (adjusted 5-year survival, 35%) (P = 0.1) (Table 3, Figure 4).

Ten of 11 recurrent tumors (91%) showed one or more chromosomal imbalances with a mean of 2.9 imbalances per tumor (range, 0 to 7). Recurrent tumors were significantly associated with a structural genetic profile with 10 of 11 recurrent tumors showing this genetic pattern. The most frequent imbalance in recurrent tumors was gain of 1q occurring in eight cases with four of these showing high-level gains of 1q. In three cases, gain of 1q was seen with concurrent loss of 10q. There were seven cases in which CGH results were obtained from both primary and recurrent tumors. Six of these showed additional abnormalities at recurrence with four tumors progressing from a balanced profile in the primary to an unbalanced profile in the recurrence. The most frequently acquired chromosome imbalances in recurrent tumors were gain of 1q (three cases) and loss of 10q (two cases). Gain of 1q detected in one primary tumor progressed to high-level gain of 1q in the recurrent tumor. Table 4 details the CGH and clinical data for the recurrent tumors.

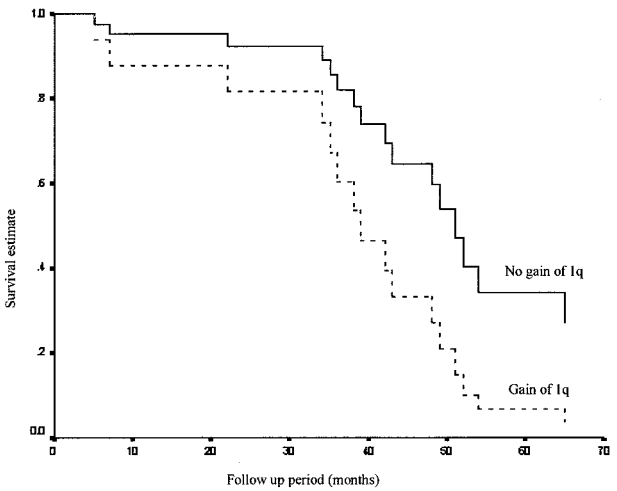

Figure 4. Adjusted survival curves for 1q status in the posterior fossa tumors from multivariate Cox proportional hazards regression analysis using histology, adjuvant therapy, extent of resection, and age (<3/>3 years) as variables. Tumors with gain of 1q are shown by a **dotted line** and tumors without gain of 1q are shown by a **solid line**. Patients receiving no adjuvant therapy, unknown adjuvant therapy, or chemotherapy alone were removed from this analysis because these groups were too small to be reliably analyzed (n = 11). Tumors with gain of 1q had a worse outcome than those without gain of 1q (P = 0.1).

Discussion

A number of informative CGH studies on ependymomas have now been reported, but none have yet correlated genetic factors with survival in pediatric tumors.[17–20] Moreover, previous studies have often considered pediatric and adult, intracranial and spinal tumors together despite the recognized differences in clinical presentation, outcome, and genetic profiles.[5,8–12,17–19] To address these issues we have analyzed a large retrospective series of archival pediatric intracranial ependymomas using a validated CGH technique. The genetic imbalances in our series of pediatric intracranial ependymomas represent one of three groups defined by the number of chromosomal abnormalities detected per tumor. These groups were confirmed by hierarchical cluster analysis. Structural tumors showed six or less chromosome imbalances, numerical tumors showed more than 13 primarily whole chromosome imbalances, and balanced tumors showed no imbalances as detected by CGH. In multivariate analysis the structural tumors had a significantly worse outcome when compared with the other two genetic groups. Importantly, genetic group and extent of surgical resection contributed significantly to outcome whereas histopathology, age, and other clinical parameters did not.

Structural tumors showed not only fewer total chromosome imbalances than numerical tumors, but also had a greater ratio of partial to whole chromosome imbalances. In neuroblastoma, tumors with primarily partial chromosome imbalances analogous to our structural group tend to have an unfavorable outcome when compared to tumors with primarily whole chromosomal imbalances.[22] This might be expected because deviation from a normal chromosome complement through unbalanced structural rearrangements is likely to have biological consequences in excess of abnormalities that maintain a broad genomic balance along individual chromosomes. Further evidence for an association of structural tumors with more aggressive behavior comes from the observation that all but one of the recurrent tumors in our cohort showed a structural genetic profile.

Overall, the most frequent chromosome imbalance in our cohort was gain or high-level gain of 1q (26%), which in turn was associated with a structural genetic profile. Gain of 1q has been reported as a common finding in intracranial pediatric ependymomas in a number of other CGH studies and is often found as a sole change or with few other chromosome imbalances consistent with our structural group.[17–20] High-level gain of 1q has been reported in a total of four primary ependymomas in two previous CGH studies. Furthermore, Kramer and col-

Table 4. Clinical Data and CGH Aberrations of 11 Recurrent Ependymomas

Case	Location	Gender	Histology	Age at diagnosis*	CGH gains	CGH losses	Genetic group
3P	PF	F	CL	58	1q	None	S
3R	PF	F	CL	86	**1q,** 13q21-q31	6q14-qter, 10q23-qter	S
4P	PF	F	CL	103	**1q,** 9p	None	S
4R	PF	F	AN	147	**1q,** 7, 9p, 15	None	S
13P	PF	F	CL	119	**1q,** 18	1p31-p35, 20q	S
13R	PF	F	CL	156	**1q,** 18	None	S
27P	PF	F	CL	31	None	None	B
27R	PF	F	CL	58	13q21-q32	None	S
28P	PF	F	CL	46	None	None	B
28R	PF	F	CL	81	1q	10q	S
30P	PF	F	CL	21	None	None	B
30R	PF	F	CL	–	None	None	B
31P	ST	F	CL	33	None	None	B
31R	ST	F	–	170	1p22-qter, 8pter-q21, 18q21-qter	9, 11pter-q13, 20, 22	S
39P	PF	M	CL	30	None	None	B
39R	PF	M	AN	–	1q	6q16-qter	S
43R	ST	M	AN	79	None	3	S
44R	PF	M	CL	54	1q	10q	S
45R	PF	M	CL	–	**1q,** 2, 8, 9	4q27q35	S

*Months; PF, posterior fossa; ST, supratentorial; AN, anaplastic; CL, classic. Gains shown in bold typeface represent high-level gains as detected by CGH (fluorescence test:reference ratio >1.5); B, balanced; S, structural.

leagues[23] also describe cytogenetic analysis of a pediatric ependymoma with a total of seven copies of part of 1q.

Not only was 1q gain a striking feature of our poor outcome structural group, but in our largest most homogenous population, the posterior fossa tumors, 1q gain itself showed a trend to poor prognosis in multivariate analysis. We also found a strong association between gain of 1q and tumor recurrence in our series. Gain of 1q was observed in three recurrent tumors from patients whose primary tumors showed a balanced CGH profile and in one other case, gain of 1q in the primary tumor progressed to high-level gain of 1q in the recurrence. A recent combined adult and pediatric CGH study also reported gain of 1q on relapse in one patient and found that gain of 1q was associated with an unfavorable outcome in univariate, but not multivariate analysis.[18] Taken together, these data strongly implicate gain of 1q and possible oncogenes in this region in the progression and pathogenesis of poor outcome pediatric intracranial ependymomas.

Gain of 1q has been shown to adversely affect survival in neuroblastoma, Wilms' tumor, and Ewing's sarcoma perhaps indicating a wide role for the involvement of 1q in the progression of pediatric tumors.[24–26] It is now important to further investigate gain of 1q as a potential marker of poor prognosis in a larger number of pediatric ependymomas treated in a standard manner. Further, although we observed gain or high-level gain of the whole of 1q in our patients, other studies have reported region-specific gains on 1q in a small number of cases. In the study reported by Ward and colleagues[20] high-level gain of 1q was restricted to 1q21 to q31 in three cases and the patient described by Kramer and colleagues[23] had seven copies of the region 1q22 to q31. Future analysis of this region may identify specific gene(s) involved in ependymoma progression.

Tumors with a numerical genetic profile had a better overall survival when compared with structural and balanced tumors in both univariate and multivariate analyses. The apparently nonrandom pattern of whole chromosome gains and losses in these tumors would be consistent with intermediate ploidy, a phenomenon that is recognized in other pediatric neoplasms. Indeed, intermediate ploidy involving specific patterns of whole chromosome gains and losses is associated with a favorable prognosis in neuroblastoma and acute lymphoblastic leukemia.[22,27] In addition, the pattern of whole chromosome imbalances observed in numerical tumors strongly resembles the predominant genetic profiles reported for adult and spinal ependymomas.[17,18] Because adult and spinal ependymomas are reported to show better overall survival than their pediatric intracranial counterparts,[5] we suggest that this numerical genetic profile may contribute to the favorable prognosis observed in a subset of pediatric intracranial tumors and adult/spinal ependymomas. In a number of adult cancers an increasing number of chromosomal imbalances has been associated with a poor prognosis, this is primarily because of the acquisition of partial chromosomal imbalances consistent with a complex karyotype and numerous chromosomal rearrangements.[28,29] The genetic profile in our numerical group is distinct from these, being characterized by whole chromosomal changes.

The overall rate of balanced pediatric ependymomas (43.9%) observed in the present study is in agreement with several smaller analyses.[17–20] These balanced tumors represent a fascinating group for further study as they are not complicated by the genetic abnormalities observed in other ependymomas. A balanced CGH profile was significantly associated with young age at diagnosis and a similar age-related difference has been noted in other pediatric series.[18] Indeed, less than 10% of adult ependymomas have a balanced profile.[17,18] This finding strongly suggests that ependymomas occurring in infants are biologically distinct from those occurring in older children and adults. However, we and others have

observed gain of 1q on relapse in children whose primary tumors were balanced,[18] which suggests that the pathway of progression in balanced tumors may be related to that of structural tumors. We further hypothesize that the tumors occurring in infants may be driven by a powerful genetic hit(s) that leads to presentation at a young age without the requirement for additional genetic changes. The as yet unidentified genetic hit(s) occurring in the balanced tumors may only exert an oncogenic effect within a specific developmental time window or cellular environment.

Several prognostic studies indicate that children presenting with ependymomas at a young age have a tendency to a less favorable prognosis when compared with older children.[2,7] Explanations for this have cited differences in resectability, location, and adjuvant therapy between the age groups. In multivariate analysis in our cohort, the extent of surgical resection and the underlying genetics of pediatric ependymomas were more important determinants of outcome than age at diagnosis.

The behavior of pediatric ependymomas is difficult to predict and treatment is currently based on clinical criteria such as age at diagnosis and extent of surgical resection. Biological factors that determine survival have been difficult to identify in ependymomas; most notably there is conflicting data concerning the relationship between histology and outcome. We found no relationship between histology and tumor behavior in our series of ependymomas, but we were able to identify genetic correlates of survival in multivariate analyses. Our study not only contributes to emerging evidence of differential chromosomal abnormalities in ependymomas dependent on age at diagnosis, but also suggests that genetic factors predict tumor behavior in pediatric ependymomas. A larger series treated in a uniform manner and studied prospectively is now needed.

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